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Transcriptional regulation of central amino acid metabolism in *Lactococcus lactis*

Rasmus Larsen

The work described in this thesis was carried out in the Molecular Genetics Group of the Groningen Biomolecular Sciences and Biotechnology Institute (Faculty of Mathematics and Natural Sciences, University of Groningen, the Netherlands).

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Transcriptional regulation of central amino acid metabolism in *Lactococcus lactis*

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To my family

Kasper, Didde, Jakob, Kirsten, Anders, Anne Mette and Torben

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Abstract

This thesis describes the functional characterisation of the transcriptional regulators GlnR, ArgR and AhrC of *Lactococcus lactis*, which are responsible for the control of genes involved in the metabolism of the amino acids glutamine, glutamate and arginine.

A chromosomal *glnR* deletion mutant was made and compared to the wild-type strain, by transcriptome analysis, during growth in either rich or poor nitrogen media. *L. lactis* GlnR was shown to repress the expression of *glnRA* (encoding the regulator itself and glutamine synthetase), *amtBglnK* (encoding putative ammonium transport and P_{II} signal transduction proteins), and *glnPQ* (encoding putative glutamine ABC transport and substrate binding proteins), in nitrogen excess. Promoter-deletion analysis and electrophoretic mobility shift assays showed that the *L. lactis* GlnR operator strongly resembles the TnrA/GlnR operator of *Bacillus subtilis*. Finally, glutamine and ammonium were shown to be the main nitrogen-effector molecules for GlnR-mediated regulation in *L. lactis*.

A random integration knockout screening identified both ArgR and AhrC as essential for the control of arginine metabolic genes in *L. lactis*. DNA microarray analyses determined ArgR and AhrC to be dedicated to the regulation of arginine metabolism, and demonstrated the effect of disrupted arginine regulation on related metabolic pathways. Using purified, His-tagged derivatives of ArgR and AhrC it was shown that AhrC does not bind to DNA, but that ArgR binds to DNA in an arginine-independent manner. Arginine-dependent DNA-binding was only obtained when the two regulators were mixed. In the presence of arginine, AhrC increased the binding of ArgR/AhrC to operators in the promoters of the arginine biosynthetic operons, but inhibited binding of ArgR to the promoter of the arginine catabolic operon. Consequently, the arginine-dependent repression of arginine biosynthesis and activation of catabolism is proposed to be mediated through direct protein-protein interaction between ArgR and AhrC in *L. lactis*.

The work described in this thesis contributes to the understanding of transcriptional gene regulation in *L. lactis*, where relatively few regulators have been characterised so far. The study illustrates the strength of combining a global

products for thousands of years, and has consequently led to the classification of *L. lactis* as 'generally recognised as safe' (GRAS).

The economic importance of *Lactococcus*, which has primarily led to research into the industrially relevant characteristics of this organism, has ultimately also paved the way for more fundamental research, culminating in the elucidation of the genome sequence of *L. lactis* subsp. *lactis* IL1403, notwithstanding that comparatively little is known about gene regulation in this organism. What is known will be reviewed here, with a focus on the transcriptional regulation of central parts of nitrogen metabolism in *L. lactis* and other low G+C Gram-positive bacteria. Considering the scope of the field, and differences between organisms, details are only presented in areas directly connected to this study, namely, the regulation of transcription initiation in the metabolism of the amino acids glutamine, glutamate, and arginine.

Regulation of transcription initiation in bacteria

Various mechanisms are devoted to regulation of cellular functions in each step on the path from gene transcription to protein degradation. The main advantage of regulation of transcription initiation is that it is energetically economical. A disadvantage is the time it takes from sensing an environmental signal to the required synthesis of the active protein. Allosteric regulation *e.g.*, where enzymatic function is directly modulated in response to the presence or absence of a specific metabolite, leads to faster responses.

In this section, definitions for a range of factors involved in regulation of transcription initiation are given for *L. lactis* specifically, as well as for bacteria in general.

Main 'players' of transcription in *Lactococcus lactis*

Transcriptional regulation affects the functions of the main 'players' of transcription: promoters, RNA polymerase (RNAP) and sigma (σ) factors. Promoters determine strength, place and time of transcription of all genes. Initiation of transcription requires the interaction between a σ factor, RNAP and the promoter, the σ factor guiding RNAP to a specific promoter. The RNAP holoenzyme (RNAP and σ

transcriptome approach with classical molecular techniques, and the care that must be taken when attempting to predict global regulatory circuits.